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Effect of High Oxygen Atmosphere Storage on Quality, Antioxidant Enzymes, and DPPH-Radical Scavenging Activity of Chinese Bayberry Fruit

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The influence of high O_2 atmosphere on postharvest decay, quality, total phenolic, total anthocyanin contents, antioxidant enzymes activity, and antioxidant activity of Chinese bayberry fruit was investigated. Freshly harvested Chinese bayberry fruits were placed in jars and ventilated continuously with air or with 80 and 100% O_2 for up to 12 days. Samples were randomly selected initially and at 3-days interval during storage. The fruit exposed to high O_2 was resistant to decay, had high levels of total soluble solids, titratable acidity and ascorbic acid contents, and also reduced the increment of pH value. High O_2 treatment was less stressful as reflected by having the significantly lower malonaldehyde contents and higher catalase, ascorbic acid peroxidase, and peroxidase activities during storage. Both 80% and 100% O_2 treaments had also retained the bioactive contents and 1,1-diphenyl-2-picrylhydrazyl radical scavenging activity during storage. These results indicate that elevated O_2 levels may improve the ability of the antioxidative defense mechanism in Chinese bayberry and result in a better control of fruit decay.

KEYWORDS: Chinese bayberry; high O_2 atmosphere; antioxidant enzymes; antioxidant activity; fruit decay

INTRODUCTION

Chinese bayberry (*Myrica rubra* Sieb. & Zucc.) is a tropical or subtropical native fruit in China with high commercial value for its red to purple color and appealing taste. To obtain the best flavor, Chinese bayberry is commercially harvested as almost fully ripe from mid June to early July. However, at this stage, the fruit is soft and highly perishable, susceptible to mechanical injury, physiological deterioration, water loss, and microbiological decay, with limiting postharvest life to 1−2 days under ambient temperature, which has resulted in a reduced market value (*1*).

Storage in lower temperature combined with conventional low O_2 controlled atmosphere storage has been reported to reduce Chinese bayberry fruit decay (*2*). However, the growth of anaerobic fungi and accumulation of anaerobic fermentation products can influence its storage life and flavor (*3*). More recently, elevated O_2 modified atmosphere has been shown to prolong the shelf life of various horticultural products (*4*−*8*). It was suggested that high O_2 treatment resulted in the suppression of microbial growth and therefore retarded decay in fruit (*8*). However, the mechanisms by which high O_2 atmosphere inhibit fruit decay are yet unclear.

Elevated O_2 concentrations can cause the production of reactive oxygen species (ROS), such as superoxide, hydrogen peroxide, and the hydroxyl radical, thus damaging plant tissues (*9*). The sensitivity to O_2 toxicity varies among plant species (*4*). Defense against oxidative stress in plants to prevent or alleviate the damage from ROS includes enzymatic ROS scavenging systems and nonenzymatic antioxidant compounds. The enzymatic ROS scavenging systems included superoxide dismutase (SOD) and catalase (CAT) (*10, 11*), the glutathione peroxidase system and the ascorbate−glutathione cycle (*12, 13*). The behavior of water and lipid soluble antioxidants, such as ascorbic acid, glutathione, α-tocopherol, carotenoids, and various types of secondary metabolites, mostly composed of total phenolics compounds such as flavones, flavonols, and anthocyanins have also been linked to function as ROS scavengers. In previous work, high O_2 atmosphere storage effectively inhibited the decay of blueberries and strawberries (*7, 14*). But it is still unknown how high O_2 ameliorates fruit decay, and there were no reports published about the effects of high O_2 atmosphere storage on antioxidant enzymes and nonenzymatic antioxidants in Chinese bayberry.

The objective of this study was to investigate the effect of high O_2 treatment on antioxidant status of Chinese bayberry in association with fruit decay and fruit quality during storage and then to understand the role of high O_2 atmosphere in bayberry fruit decay control.

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MATERIALS AND METHODS

Plant Material and Treatment. Chinese bayberry (*Myrica rubra* Sieb. & Zucc. cv. Wumei) was hand-harvested from a commercial orchard in Suzhou (June 2006), Jiangsu province, and transported to the laboratory within 4 h. Fruit was sorted to eliminate damaged, unripe fruit and selected for uniform size and color. Two kilograms of fruit were placed in a 8-L jar, and three jars were used for each treatment. The jars were placed at 5 °C and connected to a continuous flow (40 mL min^{-1}) of humidified air (control), 80% and 100% O_2 (balance N_2 in all high O_2 treatments). Oxygen concentration was checked regularly with an O_2/CO_2 analyzer (AMETEK, Pittsburgh, PA) and maintained at ± 2% during storage. Samples were taken at 3-day intervals during storage for decay evaluation. At each time point, pulp tissues were taken from each fruit, frozen in liquid nitrogen, and stored at −80 °C until analyzed.

Fruit Decay. The symptom of decay in Chinese bayberry during storage is visible mold growth on the fruit surface. Fruit decay was visually estimated by measuring the extent of decaying area on 15 fruits from each replicate and was determined by rating on a scale from 0 to 3, with 0 = normal (not decayed); 1 = slight (up to 15% of the surface affected); 2 = moderate (15−50% of the surface affected); 3 = severe (>50% of the surface affected). The fruit decay index was calculated using the following formula: $[(1 \times N_1 + 2 \times N_2 + 3 \times N_3) \times 100/(3 \times N)]$, where N is the total number of fruit measured, and N_1, N_2, and N_3 are the numbers of fruit showing different degrees of decay.

Total Soluble Solids, Titratable Acidity, and pH. Ten fruits from each replicate were wrapped in cheesecloth and squeezed with a hand press, and the juice was analyzed for total soluble solids (TSS), titratable acidity (TA), and pH. TSS was determined at 25 °C using a portable refractometer (WYT-4, Quanzhou, China). TA was determined by titrating 20 mL of bayberry juice to pH 8.2 using 0.2 mol L^{-1} NaOH, and pH was measured with a pH-meter (PHS-25B, Shanghai, China).

Respiratory Rate and Ethylene Production. Ten fruits for each of three replicates at each time point were enclosed in 250 mL glass jars at 5 °C, 10 mL of headspace gas was taken from each jar at the end of 2 h enclosure. CO_2 was measured with an infrared gas analyzer (GXH-305, Beijing, China). Ethylene production was determined by gas chromatography using a flame ionization detector (Shimadzu 14B, Kyoto, Japan).

Malondialdehyde (MDA) and Ascorbic Acid Content. To analyze MDA content, 2 g of fresh tissue was homogenized with 5 mL of 5% (v/v) trichloroacetic acid (TCA) and then centrifuged at 12,000*g* for 10 min (4 °C). The MDA content was determined by adding 2 mL of 0.5% 2-thiobarbituric acid in 15% trichloroacetic acid to 1 mL of extraction. The mixture was heated at 95 °C for 20 min, quickly cooled in an ice-bath for 5 min, and then centrifuged at 12,000*g* for 10 min to clarify the solution. Absorbance at 532 nm was measured and subtracted from the absorbance at 600 nm. The amount of MDA was calculated with an absorptivity coefficient of 155 mmol cm^{-1}.

Ascorbic acid was quantitatively determined by using 2,6-the dichlorophenolindophenol dye method as described by Jones and Hughes (*15*) with modifications. Fresh samples (10 g) were homogenized with 10 mL of 3% metaphosphoric acid (v/v). The extract was made up to a volume of 100 mL and centrifuged at 3000*g* for 15 min at room temperature. After decolorizing with 10 g of diatomite, 10 mL of supernatant was titrated against the standard 2,6-dichlorophenolindophenol dye.

Enzyme Activities. Two grams of pulp tissues was ground with a chilled mortar and pestle in 10 mL of pH 7.0, 50 mmol L^{-1} phosphate buffer or pH 7.5, Tris-HCl buffer (containing 3 mmol L^{-1} $MgCl_2$, 0.1 mmol L^{-1} EDTA, and 1% insoluble polyvinylpyrrolidone) at 4 °C. The homogenate was then centrifuged at 20,000*g* for 20 min (4 °C), and the supernatant was collected for the enzyme activity assay. Protein was measured according to the method of Bradford (*16*), using bovine serum albumin (BSA) as the standard.

SOD activity was determined by the method of Rao et al. (*17*). The reaction medium contained 50 mmol L^{-1} sodium phosphate buffer (pH 7.8), 14 mmol L^{-1} methionine, 3 μmol L^{-1} EDTA, 1 μmol L^{-1} nitro-blue-tetrazolium (NBT), 60 μmol L^{-1} riboflavin, and 100 μL of enzyme extract. Three milliliters of the assay mixture in uniform, transparent tubes was shaken and placed 50 cm below a light-bank consisting of four 30-W fluorescent lamps. The reaction was started by switching on the light; after 10 min, the light was turned off, and the absorbance by the assay mixture at 560 nm was recorded. One unit of SOD activity is defined as the amount of enzyme that inhibits the NBT photoreduction by 50% under the conditions of the assay.

CAT activity was determined according to Beers and Sizer (*18*) by monitoring the disappearance of H_2O_2 by recording the decrease in absorbance at 240 nm of a reaction mixture containing 50 mmol L^{-1} sodium phosphate buffer (pH 7.0), 12.5 mmol L^{-1} H_2O_2, and 20 μL of enzyme extract. One unit of CAT activity is defined as the amount of enzyme that decomposes 1 μmol of H_2O_2 per minute per milligram of protein under the conditions of the assay.

APX activity was measured as described by Nakano and Asada (*19*). The assay mixture consisted of 2.8 mL of 50 mmol L^{-1} sodium phosphate buffer (pH 7.0), 100 μL of 9 mmol L^{-1} ascorbic acid, 10 μL of 30% H_2O_2, and 100 μL of enzyme extract. One unit of APX activity is defined as the amount of enzyme that oxidized 1 μmol of ascorbate per minute per milligram of protein.

POD was assayed using the method of Kochba et al. (*20*) with modifications. The assay mixture (2 mL) consisted of 50 mmol L^{-1} sodium phosphate buffer (pH 6.5), 6 mmol L^{-1} guaiacol, 4.5 mmol L^{-1} H_2O_2, and 1 mL of crude enzyme extract. Increment in absorbance at 470 nm at intervals of 30 s was recorded spectrophotometrically. One unit of POD activity is defined as the amount of enzyme that catalyzes the peroxidation of 1 mmol of guaiacol per minute per milligram of protein.

Total Anthocyanin, Total Phenolic, and DPPH Radical Scavenging Activity. To prepare the fruit extract, 5 g samples from each replica were homogenized with 5 mL of precooled 95% ethanol containing 3% formic acid (v/v), and after centrifugation at 10,000*g* for 15 min (4 °C), another 15 mL of 80% ethanol containing 5% formic acid (v/v) was used to extract the residue again. The supernatant was combined to make the final volume of 25 mL for analysis.

Total anthocyanin content of bayberry extract was measured using the pH differential method (*21*). Absorbance was measured at 510 and 700 nm, respectively, in different buffers at pH 1.0 and 4.5, using $A = [(A_{510} - A_{700})_{pH1.0} - (A_{510} - A_{700})_{pH4.5}]$ with a molar extinction coefficient of cyaniding 3-glucoside of 29600. Results were expressed as milligrams of cyaniding 3-glucoside (C 3-G) equivalents per gram of fresh weight.

Total soluble phenolic content in bayberry extract was determined according to the Folin−Ciocalteu procedure (*22*). To 0.2 mL of diluted extract, 1 mL of Folin−Ciocalteu reagent and 0.8 mL of Na_2CO_3 (75 g L^{-1}) were added. The mixture was incubated at 30 °C for 60 min. For the control, 0.2 mL of ethanol was used, and the absorbance was measured at 765 nm. The results were expressed as milligrams of gallic acid equivalent (GAE) per gram of fresh weight.

The antiradical capacity of the sample extract was estimated according to the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay (*23*). Briefly, an aliquot (0.1 mL) of the ethanol extract was added to 2.9 mL of DPPH (120 μmol L^{-1}) in methanol. A spectrophotometer (UV-754, Shanghai) was used, and the absorbance at 517 nm was measured after the reaction mixtures were incubated for 30 min at 30 °C in dark. Inhibition percentage was calculated using the equation: % inhibition = $[(C - S)/C] \times 100$, where C is the net absorbance of the control, and S is the net absorbance of the sample. α-tocopherol was used as a standard antioxidant analyzed at the same time. The final results were calculated and expressed as α-tocopherol equivalents (TE) per gram on a fresh weight basis.

Data Analysis. Experiments were performed using a completely randomized design. All statistical analyses were performed using SPSS 13.0 (SPSS Inc., Chicago, IL). The data were analyzed by one-way analysis of variance (ANOVA). The treatment means were separated using Tukey's test, and differences at $P \leq 0.05$ were considered to be significant.

RESULTS AND DISCUSSION

Fruit Decay. Fruit decay was markedly affected by different storage atmospheres (**Table 1**). Chinese bayberry stored under

Table 1. Changes in Chinese Bayberry Fruit Decay, Total Soluble Solids (TSS), Titratable Acidity (TA), and pH during Storage at 5 °C in Air or High O_2 Atmosphere[a]

treatment	decay index (%)	TSS (%)	TA (%)	pH
day 0, air	0.00	9.96 ± 0.06	0.77 ± 0.01	3.53 ± 0.01
day 3, air	2.22 ± 0.85 a	9.33 ± 0.06 b	0.71 ± 0.01 b	3.56 ± 0.01 a
80% O_2	1.11 ± 0.90 a	9.63 ± 0.06 a	0.74 ± 0.01 a	3.54 ± 0.01 a
100% O_2	0.00 a	9.67 ± 0.06 a	0.76 ± 0.01 a	3.53 ± 0.02 a
day 6, air	10.00 ± 0.73 a	8.97 ± 0.06 c	0.67 ± 0.01 b	3.57 ± 0.01 a
80% O_2	4.45 ± 0.92 b	9.17 ± 0.06 b	0.71 ± 0.01 a	3.55 ± 0.01 a
100% O_2	2.22 ± 0.92 b	9.37 ± 0.09 a	0.72 ± 0.01 a	3.53 ± 0.01 a
day 9, air	21.11 ± 1.09 a	8.67 ± 0.06 c	0.66 ± 0.01 a	3.59 ± 0.01 a
80% O_2	8.89 ± 1.92 b	8.97 ± 0.06 b	0.68 ± 0.01 a	3.56 ± 0.02 a
100% O_2	7.78 ± 1.92 b	9.21 ± 0.01 a	0.69 ± 0.01 a	3.54 ± 0.01 a
day 12, air	54.55 ± 2.20 a	8.34 ± 0.07 c	0.60 ± 0.01 b	3.60 ± 0.01 a
80% O_2	22.22 ± 2.68 b	8.94 ± 0.03 b	0.62 ± 0.01 b	3.56 ± 0.01 b
100% O_2	16.67 ± 1.23 c	9.13 ± 0.06 a	0.65 ± 0.01 a	3.54 ± 0.01 b

[a] Data were expressed as the mean ± SEM of triplicate assays. Values in the same column with different letters for each day were significantly different at $P \leq 0.05$.

Table 2. Changes in Chinese Bayberry Fruit Respiratory Rate, Ethylene Production, Ascorbic Acid, and Malondialdehyde (MDA) Content during Storage at 5 °C in Air or High O_2 Atmosphere[a]

treatment	respiratory rate (mg CO_2 kg^{-1} FW h^{-1})	ethylene production (μL kg^{-1} FW h^{-1})	ascorbic acid (mg 100 g^{-1} FW)	MDA (nmol g^{-1} FW)
day 0, air	34.98 ± 1.27	23.67 ± 0.56	65.53 ± 1.30	1.64 ± 0.55
day 3, air	26.60 ± 1.01 a	21.60 ± 0.66 a	62.93 ± 1.22 a	9.64 ± 0.54 a
80% O_2	24.70 ± 0.77 b	20.63 ± 0.46 a	63.65 ± 1.20 a	7.14 ± 0.50 b
100% O_2	23.41 ± 1.85 b	19.67 ± 0.56 a	63.66 ± 1.10 a	6.03 ± 0.51 b
day 6, air	23.48 ± 1.67 a	19.82 ± 0.76 a	55.35 ± 1.00 a	12.21 ± 0.52 a
80% O_2	20.04 ± 1.15 b	17.17 ± 0.46 b	55.76 ± 1.20 a	11.20 ± 0.56 a
100% O_2	15.23 ± 1.02 c	14.37 ± 0.49 c	58.37 ± 1.30 a	10.08 ± 0.51 a
day 9, air	21.07 ± 1.72 a	17.67 ± 0.66 a	44.02 ± 0.90 b	12.84 ± 0.55 a
80% O_2	17.54 ± 1.76 a	15.97 ± 0.56 b	47.42 ± 0.74 a	11.84 ± 0.53 a
100% O_2	12.56 ± 1.70 b	13.21 ± 0.51 c	46.62 ± 1.20 a	11.29 ± 0.56 a
day 12, air	20.96 ± 1.56 a	17.34 ± 0.57 a	40.89 ± 1.40 b	16.21 ± 0.52 a
80% O_2	12.61 ± 1.51 b	12.94 ± 0.53 b	44.05 ± 0.80 a	15.62 ± 0.51 a
100% O_2	10.22 ± 1.58 b	10.13 ± 0.66 c	43.75 ± 1.20 a	14.26 ± 0.53 b

[a] Data were expressed as the mean ± SEM of triplicate assays. Values in the same column with different letters for each day were significantly different at $P \leq 0.05$.

air showed slight fungal decay on day 3 but 55% decay on day 12 during storage at 5 °C. Fruit exposed to 80% and 100% O_2 exhibited visible fungal decay on day 6, and the decay rate increased gradually thereafter. After 12 days, the decay index of Chinese bayberry exposed to 80% and 100% O_2 was only 20% and 17%, respectively. No significant difference was noted in fruit decay between 80% and 100% O_2 treatments during storage. Similar results were obtained on strawberry (*6*) and blueberry (*7*). High O_2 treatments (80−100%) were more effective in suppressing strawberry fruit decay caused by *Botritis cinerea* infection than in inhibiting the growth in vitro of *Botritis cinerea* (*7*), suggesting that the high oxygen atmospheres have an effect on the fruit itself as well as the decay caused by fungus, thereby resulting in greater effect in vivo than in vitro. However, the mechanism by which high O_2 atmosphere inhibits fruit decay is still unclear.

TSS, TA, and PH. TSS contents of Chinese bayberry in all treatments decreased with the storage time (**Table 1**). High O_2 atmosphere maintained higher TSS contents in comparison with that with air treatment. In blueberries, after storage of 28 days or longer, significantly higher values of TSS were maintained in fruit held at O_2 levels above 60% than fruit held at 40% and lower O_2 levels (*7*). In contrast, significantly lower TSS values in high O_2 treated strawberries than in air-stored fruit during the later period of storage at 5 °C were reported in earlier studies (*24*). TA contents of Chinese bayberry decreased gradually during storage corresponding to the pH increase in all treatments. Fruits stored in high O_2 atmosphere tended to have higher TA content and lower pH values than control fruit. No significant differences were observed among all of the treatments on all sampling days. Similar results were also observed by Zheng et al. (*7*) in blueberries. However, Pérez and Sanz (*24*) found significantly higher TA content before day 4 and lower TA content after day 7 in strawberry fruit exposed to 90% O_2 + 10% CO_2 than in fruit held in air during 9 days of storage at 8 °C. As the main substrates of respiratory metabolism, sugars and acids are depleted, causing corresponding changes in TSS, TA, and pH during storage. It has been shown that exposure of harvested horticultural crops to superatmospheric O_2 levels may stimulate, have no effect on, or reduce rates of respiration, depending on the commodity, maturity stage, time, and temperature of storage (*4*). The different change patterns of pH, TA, and TSS in different studies could be associated with the different effects of elevated O_2 on the commodity respiratory rate.

Respiratory Rate and Ethylene Production. The respiratory rate of Chinese bayberry measured as CO_2 evolution at harvest was around 35 mg CO_2 kg^{-1} h^{-1} (**Table 2**). During the 5 °C storage period, respiration of both high O_2 treated and control fruit decreased gradually. No significant differences were observed among any of the treatments in the first 3 days. After day 3, comparable respiratory rate was found among different high O_2 treatment groups and the air control group, and 100% O_2 was the most efficient in reducing respiration throughout the storage period. At the end of storage, respiratory rates of Chinese bayberry stored in air, 80% and 100%, were 21.0, 12.6, and 10.2 mg CO_2 kg^{-1} h^{-1}, respectively. Ethylene production of all fruit was reduced from 23.7 μL kg^{-1} h^{-1} initial to 13.5 μL kg^{-1} h^{-1} at the end of storage on average (**Table 2**). Elevated O_2 atmospheres reduced the ethylene production slightly, but significantly, from day 3 and thereafter. The improved inhibition of the respiratory rate and ethylene production in bayberries were obtained with increased O_2 concentration. The lower respiration rate and ethylene production for Chinese bayberry at 100% O_2 was possibly a contributing factor to its extended shelf life. Similar results were obtained in other previous research, in which 80% or 100% O_2 reduced ethylene production rates, delayed ripening of mature green and breaker tomatoes at 20 °C, and also 40, 60, or 80 kPa O_2 inhibited the respiration rates of Bartlett pear slices and ethylene production during 4 days at 10 °C (*4*). These data suggest that high O_2 treatments could inhibit the respiration and ethylene release of Chinese bayberry, thereby delaying the deterioration of fruit quality. This conclusion is supported by the observations that in higher O_2 treatments, there were higher TSS, TA content, and lower pH values of **Table 1** in the present work.

Ascorbic Acid and MDA. There was a decrease in the measurement of ascorbic acid content after harvest in all treatments, which indicates the nutrition loss in fruit with storage (**Table 2**). No significant differences in ascorbic acid loss were found between the control and high O_2 treated fruits. The

Table 3. Changes in Chinese Bayberry Fruit Superoxide Dismutase (SOD), Catalase (CAT), Ascorbate Peroxidase (APX), and Peroxidase (POD) Activities during Storage at 5 °C in Air or High O_2 Atmosphere[a]

treatment	SOD activity (U mg^{-1} protein)	CAT activity (U mg^{-1} protein)	APX activity (U mg^{-1} protein)	POD activity (U mg^{-1} protein)
day 0, air	899.36 ± 21.27	44.64 ± 0.96	14.26 ± 1.30	80.07 ± 5.00
day 3, air	937.40 ± 31.01 a	42.77 ± 1.06 b	26.33 ± 1.20 c	88.54 ± 3.14 c
80% O_2	960.34 ± 40.77 a	47.17 ± 0.88 a	30.30 ± 1.20 b	97.07 ± 2.11 b
100% O_2	970.39 ± 40.85 a	49.73 ± 1.56 a	36.54 ± 1.00 a	107.42 ± 6.17 a
day 6, air	644.13 ± 41.67 a	38.04 ± 0.76 b	34.81 ± 1.00 c	92.97 ± 3.31 c
80% O_2	674.52 ± 31.15 a	40.81 ± 0.96 a	40.98 ± 1.20 b	107.18 ± 2.37 b
100% O_2	723.81 ± 34.02 a	43.53 ± 1.49 a	46.10 ± 1.30 a	112.58 ± 2.37 a
day 9, air	454.69 ± 34.72 b	34.23 ± 0.66 c	26.53 ± 0.90 b	88.14 ± 2.51 b
80% O_2	550.63 ± 36.76 a	37.05 ± 1.56 b	34.67 ± 1.74 a	93.83 ± 1.97 a
100% O_2	627.55 ± 37.70 a	41.46 ± 1.51 a	31.01 ± 1.72 a	97.50 ± 1.90 a
day 12, air	614.87 ± 38.56 b	31.13 ± 0.87 b	19.82 ± 1.40 c	85.20 ± 1.50 c
80% O_2	718.66 ± 33.51 a	36.73 ± 1.53 a	23.94 ± 0.80 b	89.26 ± 1.44 b
100% O_2	675.35 ± 20.58 a	39.08 ± 1.66 a	28.55 ± 1.20 a	94.79 ± 1.27 a

[a] Data were expressed as the mean ± SEM of triplicate assays. Values in the same column with different letters for each day were significantly different at $P \leq 0.05$.

changes in MDA content were proved to be similar in all treated fruits stored at 5 °C for 12 days. MDA contents of bayberry stored in both high O_2 atmospheres and air increased with storage time, and a marked increase in MDA content of the fruits stored in air was found (**Table 2**). High O_2 atmosphere storage can inhibit the accumulation of MDA with increased O_2 concentration. Very little information is available on the effects of elevated O_2 levels on concentrations of ascorbic acid and MDA in fresh-intact and fresh-cut fruits and vegetables. Day et al. (*25*) reported that high O_2 MAP had beneficial effects on the retention of ascorbic acid and the degree of lipid oxidation. In strawberries, treatments with 60% or 100% O_2 inhibited the loss of ascorbic acid and the accumulation of MDA (*14*).

Antioxidant Enzyme Activities. SOD activity increased slightly in air and high O_2 (80% and 100%) treated Chinese bayberry within first 3 days. The enhanced SOD activity was then decreased drastically in all treatments from day 3 to day 9 and inclined slightly at the end of the storage again. There were no clear differences between SOD activity in two high O_2 treatments and control (**Table 3**). CAT activity of control fruit decreased 1.4-fold during 12 days storage, while for bayberries stored in high O_2 atmosphere, CAT activities increased within 3 days, then declined gradually with storage time. Relatively higher levels of CAT activity were found in the fruits stored in high O_2 atmosphere compared with those stored in air (**Table 3**). APX activities increased first in both high O_2 and control fruit, and then decreased gradually with a similar tendency. There were significant differences in APX activities among the air and high O_2 treatments during the whole storage (**Table 3**) period. POD activities of Chinese bayberry fruit in different storage atmospheres increased transiently and peaked at around day 6 before it started to decrease. High O_2 atmosphere could maintain significantly higher levels of POD activity than air throughout the storage period (**Table 3**).

The accumulation of ROS resulting from an altered balance between ROS production and scavenging capacities will reduce the storage quality and marketability of fruits and vegetables (*26*). MDA is considered to be an indicator of membrane lipid peroxidation induced by oxidative stress, and SOD, CAT, APX, and POD are important ROS scavenging enzymes. High O_2 (70% O_2 concentration) treatment induced SOD and CAT activities and maintained membrane integrity in peach (*27*) and loquat (*28*) fruits. Chen et al. (*14*) also reported that treatments with >60% oxygen atmosphere maintained significantly higher levels of SOD, CAT, and APX activities in strawberries and inhibited the increases of superoxide radical production, MDA content, and fruit decay. Postharvested Chinese bayberry showed senescence metabolism after 3−6 days, indicated by the increase of membrane permeability and MDA content but rapid decrease of SOD activity (*2*). In this experiment, the membrane lipid peroxidation (MDA content) in control Chinese bayberry stored at 5 °C increased gradually, and CAT activities decreased significantly, suggesting that there might be marked oxidative stress in bayberry fruit during storage. Compared with control fruit, the degree of oxidative stress in high O_2 treatments might be less serious for the significantly lower MDA contents with higher CAT, APX, and POD activities during most storage periods. These data suggest that high O_2 treatment may be helpful in maintaining Chinese bayberry fruit resistance to senescence development and decay incidence, which are associated with oxidative stress.

Total Phenolic, Total Anthocyanin, and DPPH Radical Scavenging Activity. The major phenolic and anthocyanin in different bayberry cultivars have been identified as gallic acid and cyanidin 3-glucoside (*29*). During storage, the total phenolic content in all treated fruits exhibited a slight increase during the first 3 days; thereafter, it decreased gradually during the last 9 days. There was an increase in total phenolic content with an increase in O_2 concentrations. No significant differences in total phenolic content were found during the first 6 days of storage in all treatments. However, there were significantly higher levels of total phenolic content in fruits treated with high O_2 during the following 6 days of storage compared with that in the air control (**Table 4**). Total anthocyanin content increased in the first 6 days, then declined gradually during the remaining time, and showed similar change patterns in all treatments. No significant differences of total anthocyanin content were observed in all treatments during storage periods (**Table 4**). DPPH radical scavenging activity of Chinese bayberry showed a similar change pattern as did total phenolic and anthocyanin content during storage in response to different storage atmospheres. DPPH radical scavenging activities increased within the first 3 days and then declined gradually after they reached their peak values (**Table 4**). Significantly higher DPPH radical scavenging activities were obtained in high O_2 treated Chinese bayberry from day 6 to the end of storage than in air.

Table 4. Changes in Chinese Bayberry Fruit Total Phenolic, Total Anthocyanin and DPPH-Radical Scavenging Activity during Storage at 5 °C in Air or High O_2 Atmosphere[a]

treatment	total phenolic[b] (mg g^{-1} FW)	total anthocyanin[c] (mg g^{-1} FW)	DPPH-radical scavenging activity[d] (mg g^{-1} FW)
day 0, air	8.27 ± 0.15	4.20 ± 0.17	180.5 ± 7.4
day 3, air	8.55 ± 0.75 a	4.27 ± 0.15 a	183.3 ± 2.8 a
80% O_2	8.93 ± 0.45 a	4.37 ± 0.13 a	186.2 ± 2.6 a
100% O_2	9.75 ± 0.57 a	4.36 ± 0.14 a	192.3 ± 4.1 a
day 6, air	7.39 ± 0.11 b	4.25 ± 0.14 a	168.7 ± 5.3 b
80% O_2	7.50 ± 0.11 b	4.32 ± 0.15 a	176.8 ± 4.6 ab
100% O_2	8.06 ± 0.23 a	4.36 ± 0.11 a	180.0 ± 4.4 a
day 9, air	6.12 ± 0.14 c	4.10 ± 0.12 a	155.0 ± 4.1 b
80% O_2	6.93 ± 0.24 b	4.14 ± 0.11 a	172.5 ± 2.9 a
100% O_2	7.58 ± 0.34 a	4.23 ± 0.14 a	177.3 ± 3.2 a
day 12, air	5.06 ± 0.14 c	3.77 ± 0.14 b	115.5 ± 6.5 c
80% O_2	6.03 ± 0.11 b	3.90 ± 0.11 ab	149.0 ± 2.5 b
100% O_2	6.98 ± 0.17 a	4.07 ± 0.16 a	173.9 ± 8.1 a

[a] Data were expressed as mean ± SEM of triplicate assays. [b] Data expressed as milligrams of gallic acid equivalents per gram of fresh weight. [c] Data expressed as milligrams of cyanidin 3-glucoside equivalents per gram of fresh weight. [d] Data expressed as milligrams of α-tocopherol equivalents per gram of fresh weight. Values in the same column with different letters for each day were significantly different at $P \leq 0.05$.

In blueberries, the antioxidant activity was markedly increased by 60%−100% oxygen treatments as compared with 40% O_2 treatment and air control during 35 days of storage at 5 °C. Meanwhile, the elevated O_2 between 60% and 100% also promoted increases of total phenolic and total anthocyanin content (*7*). O_2 concentrations higher than 60 kPa promoted increases in oxygen radical absorbance capacity values, total phenolics, and anthocyanins in strawberry during the initial 7 days of storage at 5 °C, but this effect diminished with prolonged storage (*30*); Pérez and Sanz (*24*) found that, in comparison with fruits stored in air, strawberries held in 80% O_2 + 20% CO_2 had significantly higher levels of total anthocyanin during the first 4 days but significantly lower levels of total anthocyanin at the end of storage. These results suggest that the effect of high O_2 atmosphere on total phenolics, total anthocyanins, and antioxidant activities in berry crops may vary depending on the commodity, O_2 concentration, storage duration, and temperature. In present study, Chinese bayberry stored in high O_2 atmosphere (80% and 100%) had little effect on total anthocyanin content. However, bayberry fruit stored in 80% and 100% O_2 atmosphere exhibited significantly higher levels of total phenolic content and DPPH radical scavenging activity compared to those in air treated fruit. Our results indicate that storage atmosphere enriched with O_2 from 80% to 100% will improve the health benefit and antioxidant status of Chinese bayberry by positively affecting phenolic metabolism to improve DPPH radical scavenging activity.

The assay of DPPH radical scavenging activity and total phenolic and anthocyanin levels measures the overall antioxidant capacity of Chinese bayberry against ROS. Enzymatic activities of ROS scavenging were enhanced by elevated O_2 level during the storage period. Interestingly, the antioxidative capacity of Chinese bayberry was also enhanced as indicated by the DPPH radical scavenging activity and total phenolic and anthocyanin level measurements. All these results indicate that the elevated O_2 level increased the ability of the antioxidative defense mechanism in Chinese bayberry in order to first control the ROS level and eventually the fruit decay severity. However, this situation was deficient after 3 days of storage when the ROS scavenging enzymes activity and fruit overall antioxidant capacity decreased and continued throughout the remainder of the storage period. These drops possibly led to an acceleration of senescence development and an increase in fruit decay incidence. Storage with 80% and 100% O_2 atmosphere seems to be just as effective in enhancing enzyme activity, total phenolic and anthocyanin levels, and DPPH radical scavenging activity in bayberry fruit.

In summary, fruit decay and quality deterioration of Chinese bayberry were reduced by both 80% and 100% O_2 treatments. This reduction was promoted with increases in O_2 concentrations. Chinese bayberry fruit treated with 100% O_2 consistently maintained the lowest fruit decay index and the highest antioxidative enzyme activities, DPPH radical scavenging activity, and total phenolic and anthocyanin contents.

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